# ULTRASTRUCTURE OF MIDGUT ENDOCRINE CELLS IN WORKERS OF STINGLESS BEE (HYMENOPTERA, APIDAE, MELIPONINAE)

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ABSTRACT

The ultrastructure of endocrine cells of the midgut of two species of stingless bees is described. These cells are basally and solitarily positioned, besides being very similar to those found in other insect orders. In *Trigona spinipes* (Fabricius, 1793) they are cone-shaped while in *T. hypogea* Silvestri, 1902, they are club-shaped. Their cytoplasm presents secretory granules and mitochondria concentrated in the basal portion of the cell, in accordance with their endocrine nature. The secretory granules may be completely filled with an electron-dense content or have a clear space between the membrane and the dense core. Lamellar bodies are present in the apical cellular portion.

KEYWORDS. Endocrine cells, midgut, stingless bee, ultrastructure.

## INTRODUCTION

Typically, the bee midgut epithelium has been reported as being formed by two cell types: digestive or columnar cells involved in the digestive process and generative cells scattered in nests along the midgut in the basal portion of the entire digestive epithelium (CRUZ-LANDIM, 1985; SERRÃO & CRUZ-LANDIM, 1995a). However a third cell type was superficially described in the midgut of *Apis mellifera* (L.) (JIMENEZ & GILLIAM, 1990). These cells were located basally and solitarily among columnar cells and distinguished by the basal packing of secretory granules. The same general structure is characteristic of the midgut endocrine cells of ten orders of insects (PRIESTER, 1971; REINHARDT, 1976; HECKER, 1977; CASSIER & FAIN-MAUREL, 1977; ENDO & NISHIITSUTSUJI-UWO, 1981; NISHIITSUTSUJI-UWO & ENDO, 1981; BROWN **et al.**, 1985). As vertebrate-like peptide hormones have been detected in the insect midgut, their secretion could be tentatively attributed to these endocrine cells

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(TAGER & KRAMER, 1980; IWANAGA **et al.**, 1981; ENDO **et al.**, 1983; BOUNIAS & DUBOIS, 1982; BROWN **et al.**, 1986).

This paper presents the ultrastructure of midgut endocrine cells in two species of neotropical stingless bees with different feeding habits.

## MATERIAL AND METHODS

The species analyzed were *Trigona spinipes* (Fabricius, 1793) which eat pollen, and the obligate necrophage species *Trigona hypogea* Silvestri, 1902.

Digestive tracts from adult workers were removed into buffered saline solution for insects, and their midgut isolated. The pieces were fixed in 2.5% glutaraldehyde in 0.1M Na cacodylate buffer at pH 7.2, washed twice in the buffer, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in Epon-Araldite resin, following usual procedures.

Ultra-thin sections were obtained with glass knives, stained with uranyl acetate and lead citrate.

## RESULTS

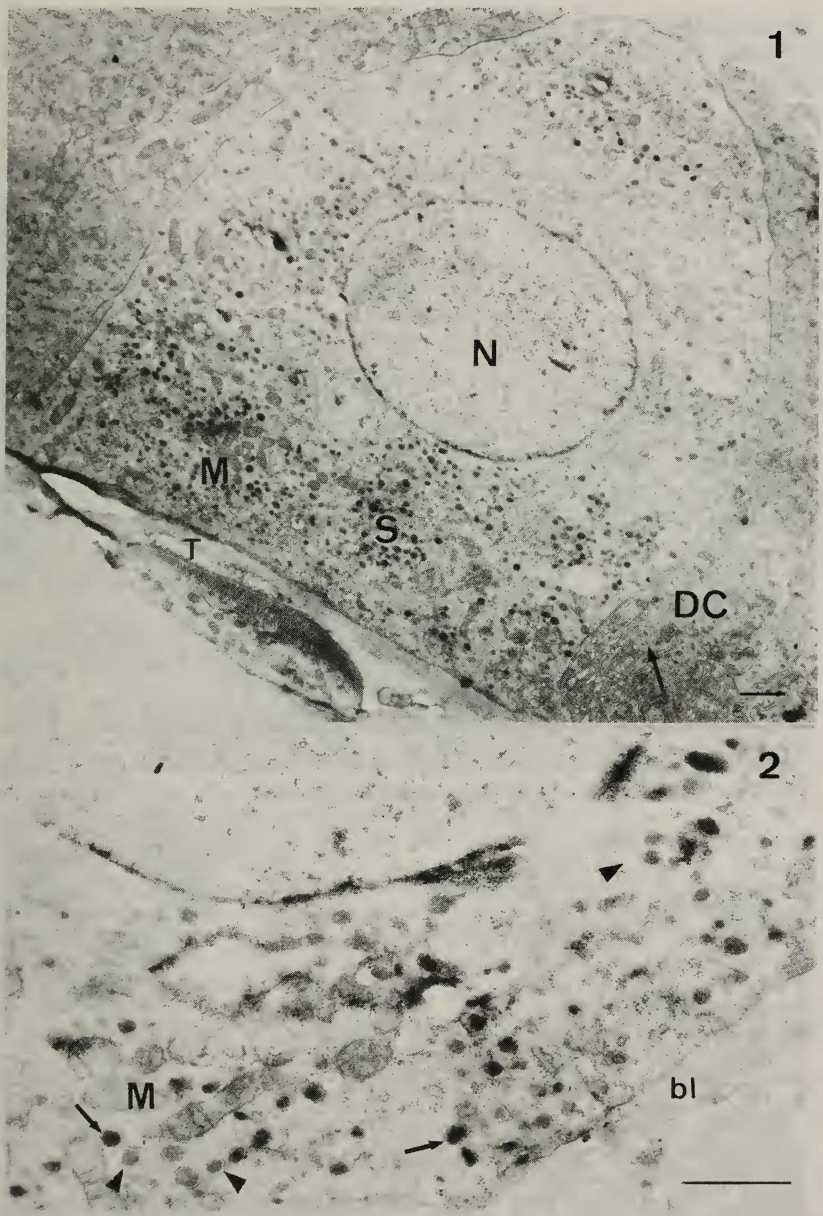
The endocrine cells were located basally among digestive cells and never reached to the midgut lumen, being more frequent in the middle and posterior midgut regions (figs. 1, 3). These cells can be distinguished from digestive cells by the absence of basal plasmic membrane infoldings and by the presence of electron-dense granules displayed toward the basal region, near the basal lamina (figs. 1, 3).

The shape of endocrine cells was different in the studied species. They were cone-shaped in *T. spinipes* (fig. 1) and club-shaped in *T. hypogea* (fig. 3). The spherical nucleus with dispersed chromatin was localized in the middle-cell portion (fig. 1). Within the cytoplasm, a rough endoplasmic reticulum surrounded the nucleus and elongated mitochondria were concentrated in the basal cell region (figs. 2, 3). The granules' electron-dense content may either fill them completely or leave a clear halo between the membrane and the dense core (fig. 2). In the apical portion, large membrane-bounded structures filled with convoluted membranous material were present (figs. 3, 4).

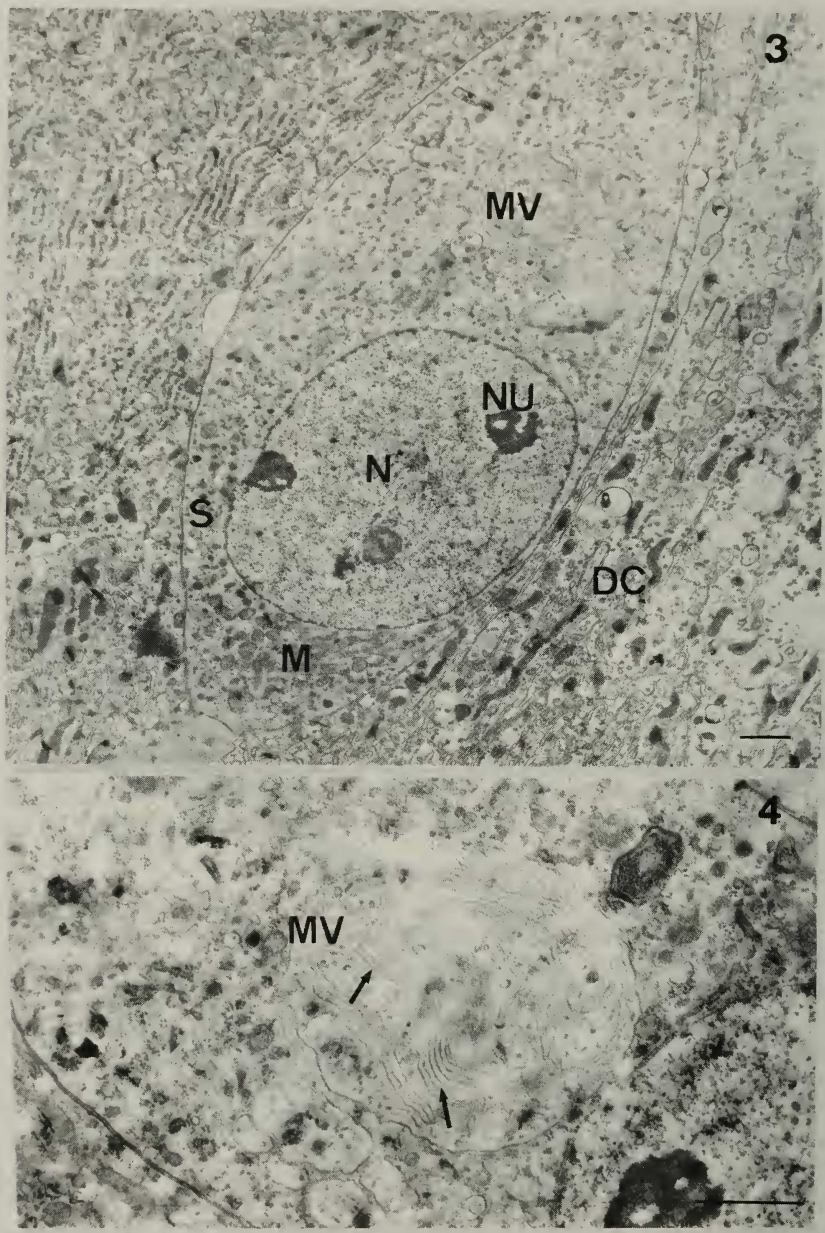
## DISCUSSION

The endocrine nature of the cells under study is being proposed only on a morphological basis. The main argument for this proposal was the isolation showed by these cells from the midgut lumen and the basal disposition of the secretory granules, wich suggest their discharge to the haemolymph.

Variations in the shape of endocrine cells were observed between *T. spinipes* and *T. hypogea*. These cells were also different, in their shape, from those reported in *Apis mellifera* (JIMENEZ & GILLIAM, 1990). Similar results were obtained by CASSIER & FAIN-MAUREL (1977) in a comparative study of fourteen insect species. In bees, each species seems to have only one type of endocrine cell. In contrast, NISHIITSUTSUJI-UWO & ENDO (1981) reported four different cellular types in *Periplaneta*. CASSIER & FAIN-MAUREL (1977) observed two types in *Japyx*, four types in *Nauphoeta* and five types in *Gryllus bimaculatus*. These authors classified endocrine cells according to cytoplasm density, granule type and size, but these differences might be attributed to different states of activity of just one cell type. The large membrane-bounded structures




Figs.1-2. Midgut endocrine cells of *Trigona spinipes* (Fabricius, 1793): 1, cone-shaped endocrine cell showing the nucleus (N),mitochondria (M) and secretory granules (S). Note the absence of basal plasma membrane infoldings (arrow) in comparison to digestive cell (DC). T. trachea. 2, detailed view of the basal portion of endocrine cell showing completely filled secretory granules (arrows) and granules with a clear space between membrane and matrix (arrowheads). bl, basal lamina; M, mitochondria. Bars: 1 µm.




Figs. 3 - 4. Midgut endocrine cells of *Trigona hypogea* Silvestri, 1902: 3, elongated endocrine cell showing basal mitochondria (M), nucleus (N), nucleolus (NU), secretory granules (S) and vesicle containing membrane-like structures (MV). 4, detailed view of vesicle (MV) containing membrane-like structures (arrows). DC, digestive cell. Bars: 1 μm.

filled with membrane-like material are very similar to the lamellar bodies present in the midgut endocrine cells of *Aedes aegypti* (BROWN **et al.**, 1985) and in the neurosecretory cells of the land snail, *Lymnaea stagnalis* (ROUBOS, 1984). These authors have pointed out lamellar bodies involved in the turnover of cell membranes that are utilized during exocytosis.

Biochemical, cytochemical and ultrastructural data have showed that endocrine cells present in the insect midgut release hormones similar to those detected from the gastric-intestinal tract of vertebrates (ENDO & NISHIITSUTSUJI-UWO, 1982; BOUNIAS & DUBOIS, 1982; RAABE, 1983; ANDRIÈS & TRAMU, 1984; BROWN **et al.**, 1986). These hormones may have effects upon the regenerative cell differentiation and enzymes production by digestive cells, as well as, upon the activity of other organs such as the fat body and the central nervous system (BILLINGSLEY & DOWNE, 1989;BROWN **et al.**, 1985). The similar ultrastructural organization of endocrine cells in the midgut of stingless bees and of other insects may indicate that these cells play a similar function in bees.

The bee workers have a diet that changes throughout their life time. As a general rule, young bees are considered to be nurse bees and have elevated pollen consumption whereas older bees are foragers and have high nectar consumption (SERRÃO & CRUZ-LANDIM, 1995b). It is possible that midgut endocrine cells release factors that control enzymes production in these different phases.

**Acknowledgments.** To Dr. Ronaldo Zucchi, FFCLRP - USP for making the specimens of *T. hypogea* available and to CNPq (300818/95-5) for financial assistance.

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Recebido em 1º.12.1995; aceito em 25.06.1996.